ADVANCES IN HEMATOLOGY

Current Developments in the Management of Hematologic Disorders

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Hemoglobin Substitutes

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H&O What are hemoglobin substitutes?

HK Because blood has multiple functions, hemoglobin-based oxygen carriers are not considered blood substitutes or even red blood cell (RBC) substitutes. RBCs themselves have many functions, one of which is to carry oxygen to tissue. To discuss research and development of hemoglobin substitutes is to discuss oxygen carriers, some of which are based on hemoglobin and others of which are not. Hemoglobin-based oxygen carriers comprise a series of proteins that are used to mimic the oxygen-carrying function of the RBC. The oxygen carrier inside the RBC is hemoglobin. For many years, researchers have known that hemoglobin outside the RBC can carry oxygen, but it is fraught with problems. The chains of the molecule itself fall apart; the molecule does not last long in the circulation; and the molecule is toxic to the kidney and other organs. The approach to using hemoglobin-based oxygen carriers as a therapy to replace that function of the RBC has entailed modifying the hemoglobin molecule.

H&O How is the hemoglobin molecule modified?

HK There have been myriad modifications of the hemoglobin molecule. For example, the chains have been linked together so they do not fall apart; the molecules have been attached to each other to create large polymers; the molecules have been modified chemically to change their affinity for oxygen; and there have been recombinant hemoglobin molecules designed to have the desired characteristics in terms of binding and releasing oxygen.

H&O What is the unmet need that hemoglobin-based oxygen carriers could fulfill?

HK Transfusions of RBCs present a variety of limitations. For example, RBCs have compatibility problems, meaning that they must be typed and the recipient must undergo a compatibility test. They transmit infectious disease and are immunogenic. RBCs are stored in a refrigerated state for a maximum of 6 weeks, after which they can no longer be used. The supply of RBCs is somewhat limited, especially for certain blood groups. Therefore, especially in emergency settings, for massive transfusions, and in the field (ie, in an ambulance or on a battlefield), the availability of an agent with the function of RBCs without these limitations would be ideal. Researchers have been trying to develop an agent that does not require crossmatching for individual patients, is minimally immunogenic, does not need to be refrigerated, could be easily transported, and could be sterilized to avoid risks of transmission of viruses and other pathogens. Because the agent would be manufactured rather than collected from volunteers, unlike blood, it would not be subject to shortages. It would be ideal if such an agent could reproduce all the functions of the RBC, but if it could only carry oxygen, it would still be very useful.

H&O How has research into hemoglobin-based oxygen carriers proceeded?

HK Research into hemoglobin-based oxygen carriers has been ongoing for over 40 years. Both human and animal hemoglobin have been used. There are three preparations under clinical investigation at present, but at least a dozen have been investigated previously. It was postulated that the hemoglobin could be removed from the RBC and modified chemically to carry oxygen in the bloodstream.

Researchers developed a series of molecules, each slightly different from the others. It was relatively easy to demonstrate efficacy in the test tube and in animal models: these various preparations did indeed carry oxygen. It was also relatively easy to demonstrate that these agents were safe in animal models, which led to a great deal of enthusiasm about researching these agents in humans. As with so many promising agents, however, there were unanticipated difficulties in the clinical development of hemoglobin-based oxygen carriers. These agents were intentionally designed so that the hemoglobin functioned much like that inside the RBC. But these very small proteins deliver oxygen in a fundamentally different way than the RBC does. These molecules go places that the RBC cannot, due to its larger size. It turns out that researchers lack an understanding of the necessary concentrations and the ideal viscosity of the preparations, and much about their function.

In terms of safety, as research progressed, adverse effects were observed in humans. The earliest agents caused renal toxicity, likely because the small molecule was directly toxic to the tissue of the kidney as it passed through it. This problem was solved by modifying the molecule. Next, it was observed that these molecules caused an elevation in blood pressure. This elevation was thought to be related to vasoconstriction of small vessels. As this condition was studied, it became apparent that this vasoconstriction was due to hemoglobin outside the RBC binding nitric oxide, which normally causes vessels to relax. There may be other explanations for this vasoconstriction leading to higher blood pressure, but hemoglobin binding nitric oxide is a major cause. Once this side effect was understood, the question arose as to whether a small increase in blood pressure was actually dangerous. In fact, the danger is not the elevation of blood pressure, which is only a signal that vasoactivity in the microcirculation is taking place. Rather, it is most likely these microcirculatory effects that are causing the harm, and mild hypertension is only an indicator. In clinical trials, myocardial infarction, pancreatitis, changes in bowel motility, and other events related to the binding of nitric oxide were observed.

Thereafter, researchers began to question whether vasoconstriction is an inevitable class effect of hemoglobin-based oxygen carriers. If not, what could be done to the molecule to prevent it? Today, researchers are attempting to discern whether it is possible to limit the binding of nitric oxide by hemoglobin through chemical or molecular-biologic means. Further questions remain to be answered as well. If the binding of nitric oxide can be prevented, researchers still need to identify the concentrations needed, how often these agents can be used, and whether the binding of nitric oxide is the sole cause of the increase in blood pressure.

The US Food and Drug Administration conducted a workshop in April on the toxicity of hemoglobin-based oxygen carriers, looking into the question of whether there is a class effect that would prevent modification of these agents to avoid the known toxicities. It was concluded that the current generation of hemoglobin-based oxygen carriers do have toxicity but it is not clear from the available evidence that it would be impossible to modify them to be safe. There may be clinical circumstances, it was further concluded, in which an agent with some toxicity would still be useful if the benefits outweigh the risks.

H&O What have researchers learned about the potential clinical application of hemoglobin-based oxygen carriers?

HK All these agents have a relatively short persistence in the circulation, with a half-life of 24–48 hours. In contrast, the RBC has a half-life of 28–30 days. Therefore, it is known that repeat dosing would be necessary if hemoglobin-based oxygen carriers were to be used in the chronic-transfusion setting, rather than only in emergent massive bleeding, meaning that these agents are probably not good candidates for this indication.

In the emergent setting, when blood is not available for casualties in the field, either in the military or in a civilian trauma setting, these agents remain viable as a focus of research. In this setting, it may be necessary to assess hemoglobin-based oxygen carriers from a risk:benefit perspective. Some agents commonly given to humans are associated with known morbidity and mortality, such as cytotoxic chemotherapies. Although such agents can cause harm, there are certain situations in which the potential benefit outweighs the harm. For example, if hemoglobin-based oxygen carriers are useful and efficacious on the battlefield, where soldiers are dying due to lack of oxygen-carrying capacity, the risk of toxicity is outweighed by the benefit these agents can confer.

Outside the emergent setting, Jehovah's Witnesses and others whose religion precludes them from receiving blood transfusions may also be good candidates for hemoglobin-based oxygen carriers in situations where they may die from lack of oxygen-carrying capacity. In addition, patients who cannot be transfused because of incompatibility, such as a sickle-cell patient with multiple antibodies or a patient with autoimmune hemolytic anemia, may be candidates for therapy with these agents. The hemoglobin-based oxygen carriers available today may not be safe enough for general use, but they may save lives in these situations. Researchers therefore must continue to attempt to improve the safety of these agents but also, in the short term, assess settings in which currently available agents should be used because the potential benefit outweighs the risk. In this regard, there is a phase II trial planned with a hemoglobin-based oxygen carrier manufactured by Biopure, and a phase III trial has recently been completed by Northfield Laboratories in the trauma setting. Other trials conducted in Europe by Sangart are awaiting data analysis.

These products are generally manufactured by small biotechnology companies. Whenever a major safety problem occurs in a trial, the community at large risks the disappearance of the research into these products due to lack of funding. All the companies are aware of the potential drawbacks of the products and, in my opinion, are eager to improve them. Unfortunately, small companies may not have the resources to invest in the needed fundamental research. Therefore, the federal government might take a leadership role in aiding private-sector research of these products.

H&O Could you discuss liposomal formulations of hemoglobin-based oxygen carriers?

HK For several years, researchers have been interested in using a lipid membrane as a way to mitigate the toxicity and lengthen the half-life of hemoglobin-based oxygen carriers. In essence, would it be possible to recreate the RBC? Liposomal drugs are already commonly used, and a great deal of research into liposomal hemoglobin occurred 10–15 years ago. At that point, the technology was in its infancy. I believe it would behoove the community to reexamine liposomal hemoglobin because of the possibility of limiting toxicity and increasing the circulation time, providing a better oxygen carrier than hemoglobin alone.

H&O What non–hemoglobin-based oxygen carriers have been investigated?

HK Hemoglobin seemed attractive as an oxygen carrier because it is naturally occurring in the cell to perform that function, but it was recognized in 1965 that perfluorocarbons could dissolve oxygen. In contrast to hemoglobin, which actively binds oxygen, these chemicals dissolve gases. It was postulated that perfluorocarbons could be used as oxygen carriers, infused intravenously into patients to bring oxygen to tissues. These water-insoluble chemicals were made soluble chemically and investigated to understand whether they could be used clinically in this manner. Unfortunately, toxicities occurred. Perfluorocarbons release oxygen differently from hemoglobin, in a linear fashion. High concentrations of oxygen are necessary to put it into solution, which means that these compounds are probably not useful in the battlefield but could be useful in the operating room. It must be recognized that because these chemicals deliver oxygen differently from blood, they cannot be used in the same way as blood. Like hemoglobin-based oxygen carriers, as small molecules, perfluorocarbons can reach parts of the body that RBCs cannot. Moreover, their toxicities are not fully understood and different compounds may have different toxicity profiles. Animal models are in use to study toxicities because early human research of these compounds resulted in activated complement and stroke. Despite these limitations, researchers have continued to pursue the possibility of using perfluorocarbons as oxygen carriers. On the positive side, unlike hemoglobin-based oxygen carriers, perfluorocarbons are cheap to manufacture. These chemicals remain attractive because they would be inexpensive, easy to store, and free of viral transmission and would not require crossmatching for blood types.

Suggested Readings

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